Is *Phytophthora cinnamomi* a Causal Agent of Oak Decline in Southern Ohio?

Annemarie M. Nagle[1], Robert P. Long[2], Laurence V. Madden[3], Pierluigi Bonello[1]

[1] Department of Plant Pathology, The Ohio State University, Columbus, OH 43210
[2] USDA Forest Service, Northern Research Station, Delaware, OH 43015
[3] Department of Plant Pathology, The Ohio State University/Ohio Agricultural Research and Development Center, Wooster, OH 44691

Abstract

White oak (*Quercus alba* L.) plays a vital ecological role and is one of Ohio's most important hardwood timber species. The decline and death of large numbers of white oaks in several southern Ohio forests during the past five years has therefore concerned foresters and land managers. The apparent concentration of mortality in low-lying areas or along waterways, and the isolation of *Phytophthora cinnamomi* Rands from the rhizospheres of declining oaks, suggest that this pathogen (a well known root disease agent) may be involved. We investigated the potential role of topography and environmental factors on pathogen activity by monitoring, monthly from July to October 2008, soil moisture, soil temperature, and soil inoculum densities in declining and healthy stands at Scioto Trail State Forest (STSF). We collected one pair of soil samples at each of three elevations along 120m transects in two declining and two healthy stands.

Both soil moisture and total isolation frequencies of *P. cinnamomi* declined throughout the summer. Inoculum concentrations were highly variable even at small spatial scales, suggesting a patchy distribution of *P. cinnamomi*, although elevation was not a significant factor. Despite such high heterogeneity in inoculum levels, declining stands had significantly higher *P. cinnamomi* propagule densities than healthy stands (P=0.021). These preliminary data provide circumstantial evidence that *P. cinnamomi* may be a contributor in this decline syndrome. These results will need to be corroborated before rational management strategies can be devised.

Figure 1. Dead white oaks in a mortality center at Scioto Trail State Forest in Southern Ohio. Mortality appeared first and was heaviest in low-lying areas, later progressing upslope. In some areas, mortality reached 80% of white oak basal area.

Introduction

- Widespread decline and mortality of mature, overstory white oak trees developed in several Southern Ohio forests from 2003 to 2005, when the forests experienced insect defoliations, development of secondary insect and pathogen attacks, and periodic excessive rainfall.
- Due to the absence of a primary environmental stressor or pathogenic component, this mortality is conceptualized as a "decline syndrome." Tree declines are typically characterized by a complex interplay of site, environmental, and secondary biotic factors.
- *Phytophthora cinnamomi* was isolated from soils at STSF in 2004. It is an invasive oomycete, known to cause severe root and bole disease on a wide range of hosts, including several members of *Fagaceae*. Pathogen propagation and dissemination is highly dependent on presence of free water in the soil.
- The patchy distribution of the decline on the landscape and the association of the heaviest decline with low elevation and watercourse areas suggest possible involvement of *P. cinnamomi* in the decline syndrome.
- Variation in disease severity across landscapes is often associated with variation in pathogen activity levels. Basic aspects of *P. cinnamomi* biology, such as inoculum distribution and dynamics are not well studied, and direct isolation from roots of dying trees is very difficult.

Primary Objectives

1. **Study *P. cinnamomi* inoculum dynamics over time and topographical gradients, and at multiple spatial scales.**
2. **Examine the relationship of *P. cinnamomi* propagule density with stand health and environmental variables: soil moisture and soil temperature.**

Figure 2. Sievings retained on a 38µm sieve (the diameter of an average *P. cinnamomi* chlamydospore) from a 60g fresh soil sample. Sievings were distributed to the surfaces of 5, 10cm diameter petri plates containing *Phytophthora* selective PARPH-V8 medium and monitored for colony formation for a week.

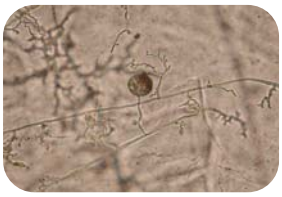


Figure 3. A chlamydospore germinates on the surface of PARPH-V8 *Phytophthora*-selective medium. Colony formation from germinating spores or mycelia was quantified to determine inoculum density of *P. cinnamomi* at each site from July to October, 2008.

Results

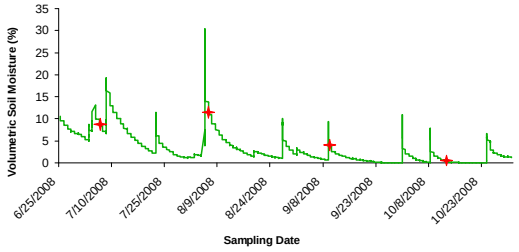


Figure 4. Average volumetric soil moisture content in Site 2 from July to October 2008, measured on a continuous basis using datalogging equipment. Red stars indicate dates at which soil samples were taken for inoculum density assays.

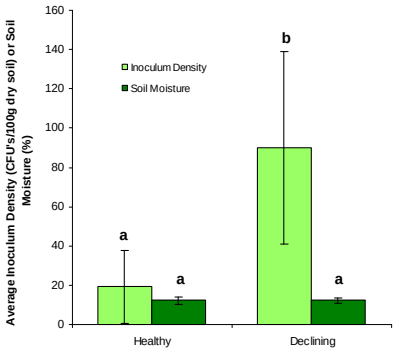


Figure 5. Average inoculum densities and soil moisture levels at STSF over the four sampling dates (July-October). Propagule densities varied widely between stands and sampling dates, but on average, declining stands had significantly higher inoculum density levels (P = 0.021) than healthy stands. Average moisture levels did not differ between healthy and declining stands, indicating that moisture levels likely do not drive differences in inoculum densities between stand types.

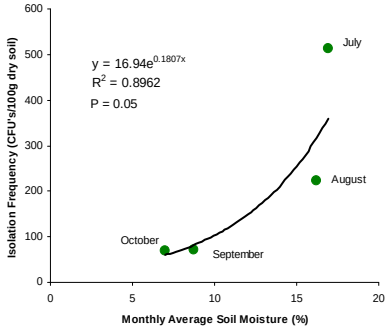
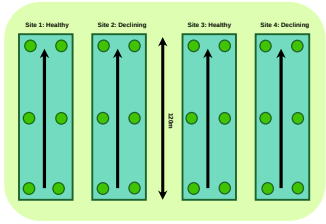


Figure 6. There was a significant (P = 0.05) positive relationship between monthly average soil moisture levels and total monthly isolation frequency of *P. cinnamomi* at STSF. Data points represent total isolation frequencies from all four study plots, and average monthly soil moistures were calculated from instantaneous measurements taken at two weeks previous to soil sampling and on the day of soil sampling.

Methodology

- 120m transects were established perpendicular to the slope contour in two healthy and two declining white oak stands at Scioto Trail State Forest in fall of 2007
- Continuous soil moisture data at one site, and continuous soil temperature data at all sites were provided by datalogger units.
- Two 10cm x 10cm soil cores, 20m apart, were taken monthly from July to October at three elevation levels on each transect (Figure 7.)
- Instantaneous soil moisture levels were measured twice monthly at each sampling point.
- Replicate soil cores were pooled, and 60g fresh soil was mixed with 200mL sterile distilled water to create a soil slurry.
- Slurry was sieved through nested 250 and 38µm sieves to concentrate *P. cinnamomi* chlamydospores and reduce contaminants.
- Material retained on the 38µm sieve was distributed to the surface of ten petri dishes containing PARPH-V8, a *Phytophthora* selective medium.
- Plates were incubated for two days, rinsed, and *P. cinnamomi* colonies counted based on morphological identification. Plates were monitored for colony development for a week.
- Representative colonies were sampled from each plate with colony development, and morphological ID's were confirmed using real-time PCR with LPV3f and LPV3r *P. cinnamomi*-specific primers.

Figure 7. Schematic of sampling transect layout at STSF. Blue boxes represent 2 declining and 2 healthy stands of white oak. Soil and soil moisture samples were taken at two points 20m apart at three elevation levels along each transect . Green circles represent sampling points along each 120m upslope transect (black arrows).



Conclusions and Discussion

- ***P. cinnamomi* inoculum levels were highly variable within sites, between sites, and between sampling dates, suggesting patchy pathogen distribution.**
- **Topography and topographically-driven soil moisture differences did not appear to affect inoculum densities within sites.**
- **However, soil moisture may drive inoculum dynamics at seasonal time scales, as evidenced by decline of total isolation frequencies with moisture throughout the summer.**
- **Declining stands had significantly higher average inoculum densities than healthy stands.**

The presence of higher inoculum densities of *P. cinnamomi* in declining stands provides circumstantial evidence that it contributes to white oak decline. This pathogen is invasive, but little is known about timing or rate of its introduction and spread in S. Ohio. Stress by native environmental factors may create a tipping point in tree physiology that is surpassed by the negative effects of a latent pathogen, resulting in decline and mortality. If *P. cinnamomi* is indeed a component of white oak decline, predictive timber salvage strategies considering environmental conditions conducive for pathogen activity would be necessary. Reproducibility of 2008 results must be confirmed before these strategies can be designed.

References and Acknowledgements

Special thanks to Dr. Yilmaz Balci for assistance with experimental design and procedures, and to Tim Fox, Shannon Quinn, Christopher Baker, and Amber Hoffstetter for their assistance in field work and laboratory assays.

Balci, Y., Balci, S., Eggers, J., MacDonald, W. L., Juzwik, J., Long, R. P., and Gottschalk, K. W. 2007. *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. Plant Dis. 91:705-710.

Long, R.P., Y. Balci, W. MacDonald, and D. Balser. 2007. White oak decline and mortality in southern Ohio. Abstract for 34th Annual Natural Areas Conference, Some assembly required: preserving nature in a fragmented landscape. Cleveland, OH.